



Northeastern University

---

Office of Environmental Health & Safety Standard  
Operating Procedures

Office of Environmental Health & Safety

---

September 25, 2006

# Standard Operating Procedures: Isolation of Corneal Stromal Fibroblasts with Collagenase

Office of Environmental Health and Safety, Northeastern University

---

## Recommended Citation

Office of Environmental Health and Safety, Northeastern University, "Standard Operating Procedures: Isolation of Corneal Stromal Fibroblasts with Collagenase" (2006). *Office of Environmental Health & Safety Standard Operating Procedures*. Paper 4.  
<http://hdl.handle.net/2047/d20003706>

This work is available open access, hosted by Northeastern University.

# ISOLATION OF CORNEAL STROMAL FIBROBLASTS WITH COLLAGENASE

## Extracellular Matrix Engineering Research Laboratory

Date: September 25<sup>th</sup>, 2006

Author: Ericka Bueno

### Reference(s):

(2000) Griffith et al., "Cornea", in Methods in Tissue Engineering, Atala, A. and Lanza, R. Eds., Academic Press, pp. 927-941.

### MATERIALS

**Note:** All materials need to be *STERILE*, instructions for 4 corneas

- Corneas, isolated as in "Dissection of Bovine Eyes" protocol.
- Fibroblast Culture medium
  - Dulbecco's Modified Eagle Medium (DMEM)
  - 10% fetal bovine serum (FBS)
  - 1% antibiotic/antimycotic SIGMA#A4668
- Phosphate-buffered saline (PBS) with 1% antibiotic
- Collagenase I solution
  - 4 mg/; Collagenase I
  - PBS (no antibiotic)
  - Prepare, warm and filter-sterilize right before use.

*For 4 corneas it is sufficient to prepare 22 ml PBS + 82 mg collagenase.*
- Petri Dishes
- Centrifuge tubes (15-ml or 50-ml)
- Surgical scalpels w/blades, forceps, pipette tips, pipette aids, absorbent pads.
- Orbital shaker.
- 1-ml sample tubes
- Trypan blue
- Hemocytometer

### PROCEDURE

1. **Sterilization:** Clean the working surface (biological hood) with disinfectant, autoclave surgical instruments, and layout all the materials outlined above within easy reach. It is recommended to wear a surgical hat and mask as well as a clean laboratory coat.
2. Dice the stroma into small pieces (1 mm<sup>3</sup>) into a sterile Petri dish and place them in a sterile centrifuge tube with 11 ml pre-warmed (37°C) collagenase solution (see "materials" to prepare and dose collagenase). Do not use cold enzyme solution, pre-warm it in the water bath. Seal the tube cap with Parafilm.
3. Bring into the incubator for 1 h. Incubation takes place under mixing; for this purpose bring a **clean (wiped thoroughly with 70% ethanol) orbital shaker** into the incubator and turn it on at low speed. **Do not turn the orbital shaker on with the digestion material on top** (if the speed setting is too high, you could kill all the cells). First set the speed, and then place the digestion tube lying horizontally on top of the orbital shaker. You can hold it in place with a small piece of lab tape to prevent it from rolling off the sides.



*Orbital shaker set-up*

4. After one hour, bring to the biological hood, aspirate the supernatant and put into a sterile tube labeled "1<sup>st</sup> hour digestate"; add 11 ml of fresh collagenase solution to the tube containing the remaining cornea chunks and label as "4-h digestate". Bring back both tubes to the orbital shaker in the incubator for 4 hours. You can aid the process by mechanically disaggregating (up and down pipetting) somewhere between the 2<sup>nd</sup> and 3<sup>rd</sup> hour.



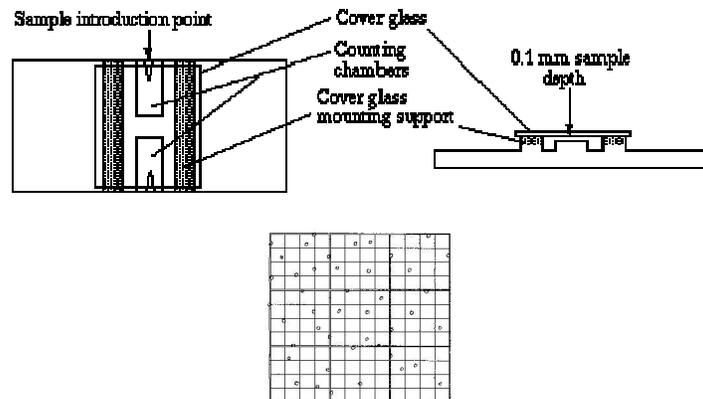
*Mechanical disaggregation*

5. Once the digestion is completed, bring back the tubes to the biological hood, use a pipette to mechanically disaggregate remaining pieces of tissue. Combine the contents of both tubes into one.
6. Wash the enzyme off by centrifuging the contents of the tube. 1,800 rpm for 12 min works well. After centrifugation you should be able to observe a cell pellet (even if small) attaching to the bottom of the tube.



*Cell pellet at the bottom of tube after centrifugation*

7. Gently aspirate the supernatant without disturbing the cell pellet. Re-suspend in fibroblast culture medium (5 or 10 ml). Mix gently by repeated pipetting, trying to dislodge the cell pellet from the walls of the tube and to create a uniform-concentration cell suspension.
8. Once you are comfortable with the uniformity of the cell suspension, take a small aliquot (0.1 ml) into a 1-ml sample tube. Place the remainder of the capped cell suspension inside the incubator.
9. Add 0.01 to 0.05 ml of Trypan blue (*ml of trypan blue*) to the 0.1 ml sample of cell suspension (*ml of cell suspension*) in the sample tube. Pipette up and down three times. Inject 10 micro liters of this mixture into each one of the two chambers of a hemocytometer (cell counting chamber, see figure below).
10. Under 10x magnification (light microscopy) count the cells inside the grids on each one of the chambers. All cells look round. Dead cells are black (they have absorbed the dye) while alive cells are white or light-bluish.
11. **COUNTING PROCEDURE:** The counting area is a central square bounded by three lines on all sides. Inside this central square there will be 25 smaller squares, bounded by single lines. Outside this central square there are 8 additional squares of similar dimensions but with less grids. Each square (including the central one) covers a 1mm<sup>2</sup> area, and its volume equals 0.1 mm<sup>3</sup> (see figure below). Count live cells (#live) and dead cells (#dead) separately. Count as many squares as necessary to make for at least 100 cells in each chamber (note the number of squares you used to count). Verify this count by counting the second chamber, and if the measurements agree, proceed to calculate the average count. If the results disagree, clean the hemocytometer with ethanol, dry thoroughly and repeat.



9 squares, each one 1 mm<sup>2</sup> in area. Grids are not accurate in this cartoon.

12. **CELL DENSITY CALCULATIONS:** The density of the cell suspension (cells/ml) and the cell viability can be calculated as follows:

a.  $\rho_{\text{suspension}} =$

$$\frac{\# \text{ Live}}{(\text{number of counted squares}) * (0.1 \text{ mm}^3 / \text{square})} \times \frac{\text{ml. of trypan blue and cell suspension}}{\text{ml. of cell suspension}}$$

b. Cell Viability (%) =  $\frac{(\# \text{ live})}{(\# \text{ live} + \# \text{ dead})} \times 100$

*Note: If the cells are less than 90% viable, the enzymatic digestion procedure may have been excessively aggressive for them.*

13. After determining the cell density in the cell suspension, cells are ready to plate to start the cell culture (see respective protocol).

## REAGENT VENDORS

**(Include catalog numbers)**

1. DMEM: Invitrogen, Fisher
2. FBS: Invitrogen
3. PBS: Invitrogen
4. Collagenase I: Sigma
5. Trypan Blue: Sigma